

# Determination of Acid Phosphatase Activity in Canned Hams as an Indicator of Temperatures Attained during Cooking

## SUMMARY

The residual acid phosphatase activity in 65 cured, canned and heat-processed hams was related to the maximum internal temperatures attained during cooking. Temperatures within the range 59.4 to 70°C (139–158°F) were indicated by the equation  $T = 74.75 - 5.10 \log \text{acid phosphatase activity}$ , standard deviation,  $\pm 1.46^\circ\text{C}$ , and that there would be no greater than a 5% chance that a calculated temperature would be more than 2.44°C above the temperature actually attained. The method lacked accuracy when applied to hams processed to higher internal temperatures. Based on these results it can be concluded that practical use can be made of the method provided that 70°C (158°F) is the highest temperature of interest although improved precision is desirable.

## INTRODUCTION

In quality control and regulatory work there is a need for an easily applied accurate laboratory method to determine the temperatures to which heat-treated meat products have been processed. One important application would be in analyzing products to be imported into the United States to ensure that the processing temperatures applied have been high enough to meet regulatory requirements. In the case of domestic products also, there are regulations which require heating to, or above, specified temperatures. Quality control laboratories in industry should often make similar determinations, both to assure compliance with regulations and to maintain quality standards. Efforts to improve the method presently employed or to develop a new, more satisfactory one have been made by Körmendy et al. (1960, 1967), Lind (1966), Cohen (1966), Višacki et al. (1967), Suvakov et al. (1967) and Gantner et al. (1968).

The meat processing industry heat-processes to various specifications. In this country the Meat Inspection Division requires that pork products cooked to eliminate trichinae attain an internal temperature (IT) of 58.3°C (137°F).

In the case of smoked ham, com-

mercial practice is to heat to approximately 60°C (140°F, IT) to produce a tendered product that is intended to be further cooked before serving. Ready-to-eat smoked meats are heated to temperatures in the range of 65.6 to 73.9°C (150–165°F, IT). Pickled sausages reach approximately 65.6°C (150°F, IT); franks, 71.1°C (160°F, IT); ready-to-eat bologna, 65.6°C (150°F, IT); and cooked ("boiled") ham, 61.1–65.6°C (142–150°F, IT). Most processors cook canned hams to 65.6°C (150°F IT) in order to obtain flavor and firmness. Review of the above list of products shows that the temperatures used or required lie in the range of 58.3–73.6°C (137–165°F).

The temperatures required in cooking canned products acceptably for importation into the United States are aimed at excluding infectious agents. The lethal effect of temperatures varies with the combined effect of time and temperature. Consequently, regulatory agencies require adequate temperatures in the light of the particular processing techniques. At present, processing tends towards relatively high temperatures and a reduction of cooking time.

Considering the interests of both processors and regulatory agencies, accurate analytical determination of processing temperatures is required from 58°C (137°F) upwards. Development of an analytical method applicable to determination over the entire range would obviously be preferable.

A method involving the measurement of residual acid phosphatase as a means of determining the temperature to which hams have been heated has been tested by Lind (1966). Results of the investigations indicate that the maximum internal temperatures of canned hams were estimated not more than 1.07°C above actual processing temperatures in the region 65–70°C (149–158°F), which lies in the middle of the temperature range. In contrast, Suvakov et al. (1967) tested the method and concluded that it yields low correlations.

The report of Körmendy et al.

(1960) indicates that the disappearance of the enzyme in heated meat is not only influenced by temperature, but also to some extent by time of cooking, pH, and content of NaCl. However, variation of time of cooking and NaCl content is limited by practical considerations and variation of pH primarily by natural considerations. If needed, compensatory corrections could be developed.

The present work was undertaken to obtain an evaluation of the accuracy with which processing temperatures can be predicted on the basis of residual acid phosphatase activity, without correction for modifying factors, on commercially cured and canned hams which were cooked at accurately measured temperatures in the range 59.4–73.6°C (139–165°F) in pilot plant equipment.

## EXPERIMENTAL

**Sample preparation.** Fresh hams were cured for 3 days at either of two local processing plants. Arterial and stitch pumping methods were used to inject 10% by weight of 68° salometer pickle and the hams were covered with pickle of the same strength. The curing temperature was approximately 45°F. The hams were boned, trimmed free of external fat, and placed in size 709 × 1010 × 404 pear-shaped cans; 1 tsp of powdered gelatin was added, the meat was compressed, and the cans were sealed under vacuum. Thermocouple receptacles were previously centered and attached to an opening in the flat end of each of the cans.

Prior to sealing the cans, custom-made 1/16-in. stainless steel, copper-constantan thermocouples (O. F. Ecklund, Barrington, Ill.) were inserted into the hams through the receptacles. The thermocouples and receptacles were sealed with gaskets. The thermocouples used (4½ in. long) reached the geometric center of the cans which has been found to be in the area of slowest temperature rise (Schack et al., 1959).

The sealed, uncooked hams were brought to the laboratory and placed

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Table 1. Residual acid phosphatase activity in canned hams cooked to different internal temperatures.

Plant I			Plant II		
Temp. °C	Acid phosphatase activity μmoles/kg ham	Program of heating <sup>1</sup>	Temp. °C	Acid phosphatase activity μmoles/kg ham	Program of heating <sup>1</sup>
59.4	254	F	68.3	32	H
59.4	310	F	68.9	27	H
60.0	229	F	68.9	17	H
60.6	258	F	69.2	10	H
60.6	287	F	69.4	24	H
62.8	178	G	69.4	41	H
63.9	100	G	69.7	41	F
64.4	168	G	70.0	3	F
64.7	46	B	70.6	30	F
65.6	94	G	70.6	37	G
65.6	47	G	70.6	9	G
65.6	33	B	71.1	20	G
65.6	24	B	71.4	15	F
66.1	33	G			
66.1	70	G			
66.1	52	G			
66.1	19	B			
66.7	14	B			
66.7	38	A			
66.7	21	A			
66.9	22	A			
66.9	27	A			
66.9	55	A			
67.2	122	G			
67.2	35	G			
67.2	27	A			
			61.4	340	C
			61.7	331	C
			61.7	232	C
			62.2	283	C
			62.8	268	C
			62.8	234	C
			64.2	206	C
			64.2	121	D
			64.4	100	D
			64.4	127	D
			65.0	89	D
			65.0	78	D
			65.3	117	C
			65.3	123	C
			65.3	147	C
			65.6	103	C
			65.8	118	C
			67.8	20	D
			68.3	31	D
			68.3	46	D
			68.6	16	D
			68.6	24	D
			71.9	31	E
			72.5	32	E
			72.5	22	E
			73.6	20	E

<sup>1</sup> Water temperature in kettle and approximate time to reach maximum internal temperature: A, 68.9°C, 450 min; B, 74.4°C, 285 min; C, 78.9°C, 200 min; D, 79.4°C, 235 min; E, 80.3°C, 285 min; F, 83.9°C, 285 min; G, 84.4°C, 205 min; H, 85.6°C, 205 min.

on processing racks. Thermocouple extension connectors were attached to the thermocouple receptacles and plugged into a 24-point recording potentiometer calibrated at 0°C with an ice water bath and at 100°C with boiling water. The processing racks with the cans of ham and connected thermocouples were lowered into a 200-gal steam-jacketed stainless steel kettle. The water temperature was controlled with a thermostatic steam valve capable of maintaining temperature in the range 70–90°C (158–194°F), to within ±1°C, as desired. The water was stirred with an industrial-type stirrer (Lightnin Mixer) to insure good circulation. Stainless steel kettle covers were used to reduce heat loss.

Sixty-five cans were cooked by heating batches of 4 to 14 cans at different water temperatures and for different periods. At internal temperatures of from 3 to 8°C (5–15°F) below the desired temperatures the cans were removed and cooled in running water held at 20°C (68°F). The center temperatures continued to rise for a period of up to 30 min as the cans were being cooled. The maximum internal temperature attained in each can was recorded and the cans were stored at 3°C (37°F) until analyzed for residual acid phosphatase activity.

Samples of 1 × 2 × ¾-in. size weighing approximately 25 g were removed from the hams at the central areas which had surrounded the ther-

mocouple tips. The samples were trimmed free of all but interstitial fat, ground three times through a 3/16-in. plate in a cold room at 3°C (37°F), and then placed in a polyethylene bag and thoroughly mixed.

**Enzyme determination.** The analytical method employed was essentially that described by Körmeny et al. (1960). The samples were weighed to 1.000 ± 0.005 g using Teflon weighing paper to prevent absorption of the liquids from the meat and then transferred to 25-ml Erlenmeyer flasks containing 4 ml of buffer (0.05M citrate buffer, pH 6.10 ± 0.05). Each sample was prepared in quadruplicate. To one flask of each set of samples 2 ml of TCA (trichloroacetic acid, 20%) were added to provide a blank. The samples were equilibrated for 5 min in a water bath-reciprocating shaker at 37.5°C. Two ml of 0.01M disodium phenyl phosphate solution were added to each flask, including the blanks. The water bath-shaker was adjusted to agitate the reactants for exactly 60 min. The reaction was stopped with the addition of 2 ml of TCA (omitting the sample blanks already containing TCA).

Instead of filtration, as in previous use of the method, the solutions were decanted into 15-ml round bottom centrifuge tubes and then centrifuged either in swing-out buckets at 1250 × g for 10 min or in an angle-head centrifuge at 7800 × g for 10 min. Three-

ml aliquots from each centrifuge tube were added to 3 ml of 0.5M sodium carbonate solution in a 16 × 150 mm test tube. The solutions were thoroughly agitated with a Vortex tube-mixer and 0.1 ml of 2,6-dibromoquinonechlorimide solution (40 mg in 10 ml absolute ethanol, stored in an amber bottle and made up 15 min prior to use) was added and mixed. The reactants were stored for 30 min in a light-tight storage box painted flat black on the inside, which contained test tube racks. The samples were read at 610 mμ.

The reactions involved are the action of acid phosphatase in splitting the substrate, disodium phenyl phosphate, into phenol and disodium monohydrogen phosphate, and the subsequent reaction of phenol, in an alkaline medium, with 2,6-dibromoquinonechlorimide. Standard curves were prepared based on the absorbances of standard solutions of phenol. The acid phosphatase activity in samples was calculated as follows:

$$(A_s - A_b) \times F \times \frac{1000}{0.0625} = \mu\text{mole}$$

phenol/kg meat

A<sub>s</sub> = Absorbance of the sample

A<sub>b</sub> = Absorbance of the blank

F = (μmole phenol/ml) per absorbance unit from standard curve

The factor 0.0625 corrected for sample dilution

A sample-dilutor apparatus (Warner Chilcott Auto-spenser) was used to dispense the buffer, substrate, sodium carbonate and sample with a precision of better than ±0.5%, thus eliminating the need for pipets and yielding accuracy with speed. The 2,6-dibromoquinonechlorimide was dispensed in a 2.5-ml syringe utilizing a ½th total volume Hamilton repeating dispenser. A 1-cm flow-through cell attached to a GME (Gilson Medical Equipment) transferator vacuum dispenser was used with a Gilford Model 2000 recording spectrophotometer: based on its capabilities, determination of absolute absorbance within .005 units should be obtainable, which, in present work, was equivalent to 3.7 μmoles phenol/kg ham.

## RESULTS AND DISCUSSION

Table 1 shows the activity of acid phosphatase in the hams expressed as μmoles phenol released/kg meat, the conditions under which the hams were cooked, and the maximum temperatures attained. The results of statistical analyses of the predictability of internal temperatures based on residual

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Table 2. Relation between acid phosphatase activity and internal temperature reached in cooking hams.

Estimation of internal temperature, T							
Group	Number of hams	Internal temperature, range °C	Regression equation	S.D. °C	Correlation		Maximum error, P = .05 °C
					coefficient (r)	sig.	
All	65	59.4 to 73.6	76.77 — 5.96 log AP	±1.92	—0.82	.01	3.20
I	9	70.6 to 73.6	70.75 + 0.66 log AP	±1.14	+0.12	N.S.	2.16
II	38	64.7 to 70.0	71.53 — 2.86 log AP	±1.22	—0.63	.01	2.07
III	18	59.4 to 64.4	79.96 — 7.62 log AP	±1.22	—0.74	.01	2.13
I and II	47	64.7 to 73.6	73.94 — 3.94 log AP	±1.97	—0.56	.01	3.31
II and III	56	59.4 to 70.0	74.75 — 5.10 log AP	±1.46	—0.85	.01	2.44
II and III (Plant A)	34	59.4 to 70.0	74.99 — 5.43 log AP	±1.69	—0.83	.01	2.86
II and III (Plant B)	22	59.4 to 70.0	76.12 — 5.53 log AP	±0.80	—0.94	.01	1.67

activity of acid phosphatase are given in Table 2. A previous report (Lind, 1966) states that the relationship approaches linearity in the range 65–70°C (149–158°F) when expressed in the form of the general equation,  $T = a - b \log$  acid phosphatase activity. To obtain information on the extent to which the present data conformed with this tendency they were analyzed over parts, as well as the whole of the range of temperatures 59.4 to 73.6°C (139–165°F).

Over the whole range, correlation of temperature with log acid phosphatase activity (log  $\mu$ mole phenol released/kg meat) was found to have the coefficient,  $r = -0.82$ , and a standard deviation (S.D.) of  $\pm 1.92^\circ\text{C}$ . The results of a one-tailed  $t$ -test based on the data indicate that an estimate of temperature would not exceed actual internal temperature by more than  $3.2^\circ\text{C}$  at the 95% level of confidence.

The scatter of data plotted for those hams processed to temperatures in excess of  $70^\circ\text{C}$  (Fig. 1) shows that they have a low correlation with data obtained at lower temperatures. The data were analyzed as Groups I, II and III consisting of hams which had been heated to within the ranges 70.6 to  $73.6^\circ\text{C}$  (159–165°F), 64.7 to  $70.0^\circ\text{C}$  (148.5–158.0°F) and 59.4 to  $64.4^\circ\text{C}$  (139–148°F), respectively.

The regression of temperature for Groups I, II and III had standard deviations of  $\pm 1.14$ ,  $\pm 1.22$ , and  $\pm 1.22^\circ\text{C}$  and yielded estimates of temperature not exceeding actual processing temperatures by more than 2.16, 2.07 and  $2.13^\circ\text{C}$ , respectively ( $P = .05$ ). However, the correlation coefficient for Group I,  $r = +0.12$ , was low and its correlation was not significant, in contrast with the parameters for Groups II and III of  $r = -0.74$  and  $-0.63$ , respectively ( $P = .01$ ). Combining the data for Groups I and II showed the correlation coefficient to be relatively low ( $r = -0.56$ ) and the standard deviation high. These results indicate that the accuracy of the method

decreased on application to hams processed to temperatures higher than  $70^\circ\text{C}$  (158°F). The result of analysis of combined Groups II and III, which included hams cooked in the range from  $59.4^\circ\text{C}$  to but not higher than  $70.0^\circ\text{C}$  (139–158°F), produced improved statistical parameters,  $r = -0.85$ , standard deviation,  $\pm 1.46$ , and maximum error,  $2.44^\circ\text{C}$ , on applying a one-tailed test,  $P = .05$ .

Fig. 1 shows the actual internal temperatures in all 65 hams plotted against residual acid phosphatase activity, expressed as  $\mu$ moles phenol released/kg meat. The regression shown,  $T = 74.75 - 5.10 \log$  acid phosphatase activity, however, is that calculated for only those 56 hams comprising Groups II and III. The data are tabulated so that hams can be dis-

tinguished according to the plant which supplied them. Individual analyses of these data (Table 2) indicate that substantially equivalent results were obtained on processing and analyzing hams from the two different sources.

The results of the present investigation are in general agreement with those of Lind (1966). Their results were the basis for a relationship,  $T = 77.3985 - 5.7109 \log$  acid phosphatase activity, standard deviation,  $\pm 0.6349$ , with which temperature in the range 65– $70^\circ\text{C}$  was determined with  $1.07^\circ\text{C}$  the maximum error with which estimated temperature might exceed the actual at the 95% level of confidence, as compared with  $2.44^\circ\text{C}$  obtained from present results. It is possible that our maximum error of  $2.44^\circ\text{C}$  was due primarily to the relatively

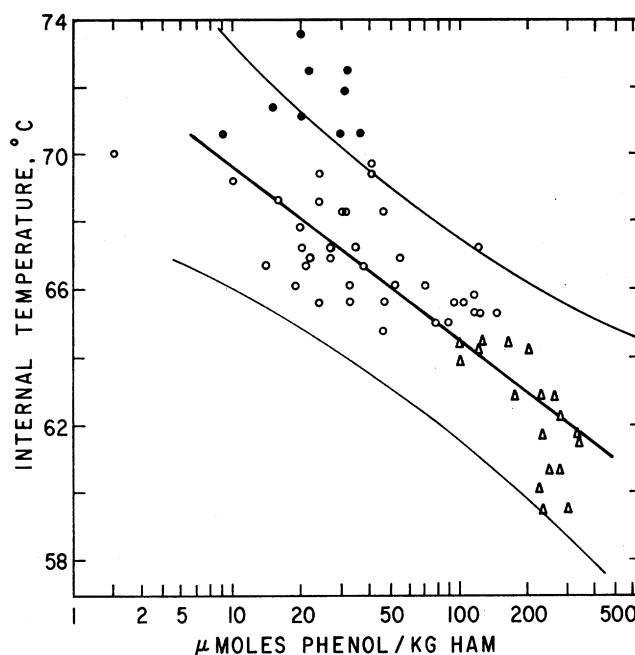


Fig. 1. Linear regression curve for relationship of acid phosphatase content of canned hams to maximum internal temperatures between  $58.4$  to  $70.0^\circ\text{C}$ : ●—data for Group I heated within range of  $70.6$  to  $73.6^\circ\text{C}$ , but omitted in calculating curve; ○—data for Group II heated within range  $64.7$  to  $70.0^\circ\text{C}$ ; △—data for Group III heated within range of  $59.4$  to  $64.4^\circ\text{C}$ . Also shown are the 95% confidence limits.

broad range of processing conditions utilized in the present work. In practical use, the significance of this is only that a larger possible error would be taken into account, i.e., an acid phosphatase activity of 20  $\mu$ moles would indicate that at the 95% level of confidence an internal temperature of at least 65.6°C (150.0°F) was attained, rather than an internal temperature of at least 66.9°C (152.5°F) as would be indicated if the maximum error were 1.07°C (1.93°F).

The poor results obtained in applying the method to hams processed at temperatures exceeding 70°C (158°F) appears of considerably greater significance, because conducting analyses on hams heated to these temperatures can be required in comprehensive control and regulatory work. The linear regression shown in Fig. 1 indicates that levels of 10  $\mu$ moles phenol/kg meat or less should have been found in hams cooked to over 70°C, while ac-

tivity ranging approximately 10 to almost 40  $\mu$ moles was actually found. The possibility exists that hams contain some of a type of phosphatase which is unusually heat resistant. However, inaccuracy in estimating low levels of phosphatase activity is inherent in the method, inasmuch as it involves measuring the absorbance produced by the phenol and 2,6-dibromoquinone-chlorimide reaction against a comparatively high blank.

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